SEUVADIS

ANNUAL MEETING 2011

Toulouse, France

28-29 November 2011

DELEGATES' GUIDE

Presentations with "title between quotation marks" are selected from the best abstracts submitted for this meeting. You'll find the abstract of posters and presentations on p.6. ***Note for presenters****: A PC will be available in the Meeting room. Please make sure that you give a copy of your presentation to Gabrielle before the end of the meeting, after removing any confidential information. The presentations will be shared within the Consortium through the Intranet. All dinners will be paid by the coordinator.*

Grand Hôtel d'Orléans - **Salle Riquet**
72, rue de Bayard - 31000 TOULOUSE
Tél. +33 5 61 62 98 47

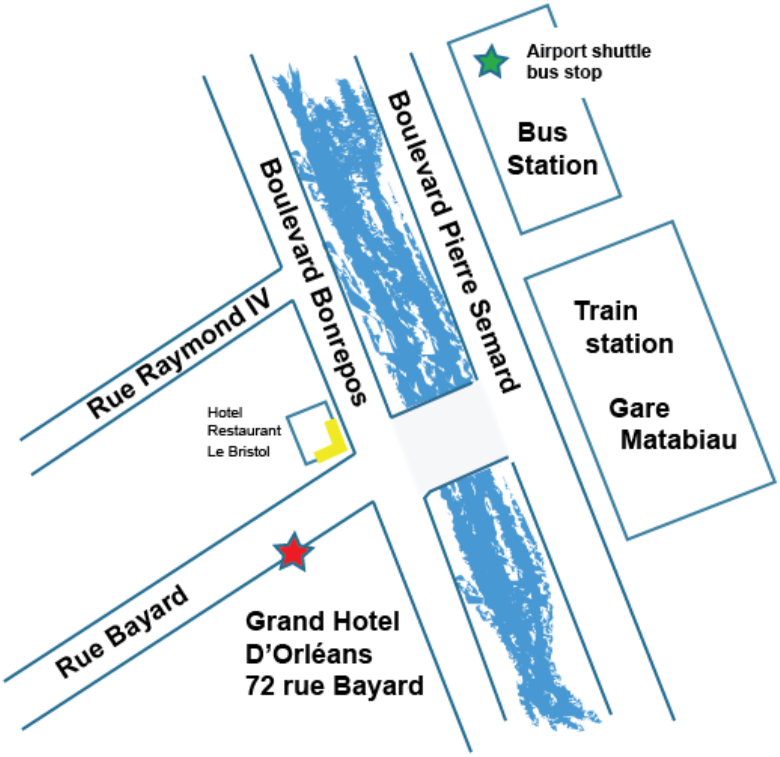
Access the meeting location:

From the Airport: The 'Navette Aéroport', the airport shuttle bus will take you directly from the airport to the bus station - Gare routière located 2 mins walk from the hotel - cf map below- Bus departs from Ground floor gate C, arrival levels every 20 mins. Journey lasts 20 to 45 min. depending on traffic conditions. (Price: 5€)

From the train station: walking distance. Cross the Canal, the hotel is located in the begining of rue Bayard. (At the entrance of rue Bayard, on the right, you'll see a yellow sign for the Hotel Restaurant Le Bristol) Le Grand Hotel d'Orléans will be on your left hand. (blue sign n°72).

By car: From Carcassonne, Narbonne, Montpellier: A61: At the exit of the A61, take exit "Albi / Bordeaux / Paris," Périphérique extérieur, exit 15 "La Roseraie." Follow the signs for "Centre Ville". Once you crossed the bridge over the railroadtracks, take the wide avenue Jean Jaures. At the end on your right, take the Boulevard de Strasbourg, the Rue Bayard is the third street on the right, direction "Gare SNCF Matabiau." Our hotel is at the end of the street, at n°72 on your right.

Closest Metro stations:
Lign A: Marengo sncf - Lign B: Jeanne d'Arc



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GEUVADIS Annual Meeting agenda:

Sunday 27th November 2011

20:30 Welcome Dinner at the Grand Hotel d'Orléans. See menu p.11

Monday 28th November 2011

9:00	WP1 Coordination and Management update	**G.Bertier, X. Estivill**
9:30	WP2 update **:** Quality control of sequence data	**AC. Syvänen, M. Brännvall**
10:00	WP3 update: Data storage, access and exchange	**N. Kurbatova**
	10:30 Coffee break	
11:00	WP4 update**:** Handling analysis and interpretation of RNA Sequence data and other functional datasets	**T. Lappalainen**
11:30	WP5 update**:** Biological and medical interpretation of sequence data for rare variants	**T. Meitinger**
12:00	WP6 update: Ethical, Legal and Social Issues of phenotype prediction from sequence variation	**GJ. Van Ommen**
12:30	WP7 update**:** Dissemination and training	**A. Cambon Thomsen**
	13:00 Group Picture - Lunch	
14:30	Sub-Projects Update: Parkinson's disease	**X. Estivill**
14:50	Sub-Projects Update: Intestinal inflammatory disorders	**P. Rosenstiel**
15:10	Life technologies and Geuvadis	**J. Mangion**
15:30	RNAseq analysis plan	**T. Lappalainen**
16:00	General discussion	
	16:30 Coffee break	
17:00	"Ontology development for annotating high throughput sequencing experiments"	**N. Kurbatova**
17:20	"Epistatic selection between coding and regulatory variation in human evolution and disease"	**T. Lappalainen**
17:40	Feedback on the project	**P. Kwok**

20:30 Dinner at Restaurant La Boheme 3 rue Lafayette +33 5 61 23 24 18
Access details p.12

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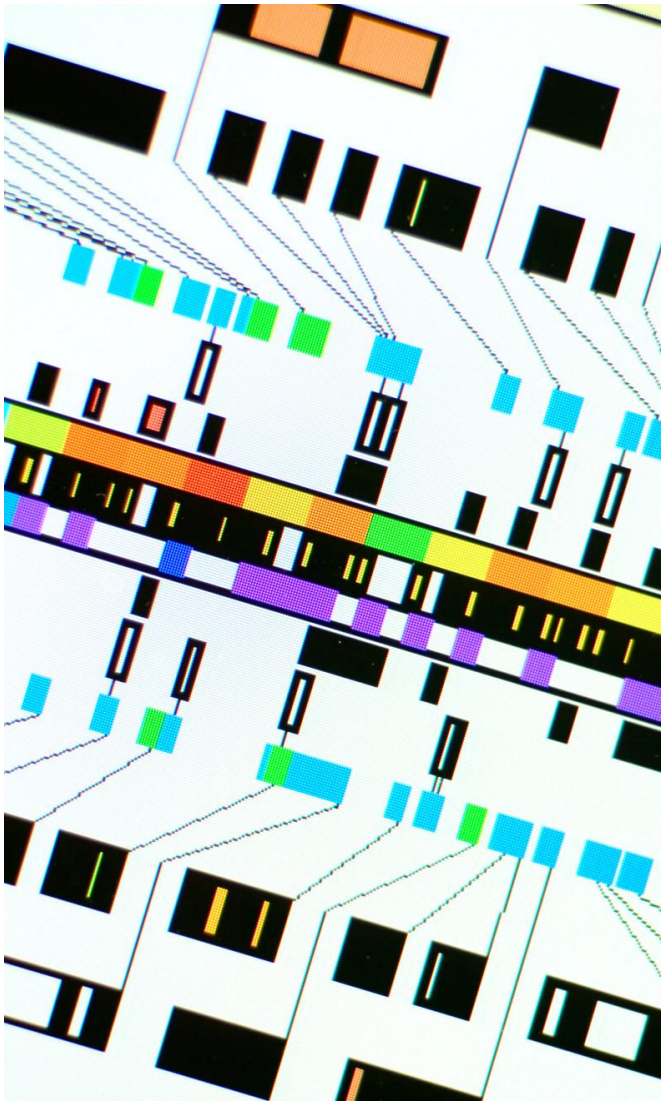
Tuesday 29th November 2011

09:00 - 13:00

Workshop: Medical applications of Next Generation Sequencing technologies.
Chair: Thomas Meitinger



Sergi Espada - © CRG

Agenda

9:00-10:30

Medical sequencing	**P.Y Kwok**
Cutting edge methods example amplicon sequencing	**J Häger**
"Next-generation sequencing into the diagnostic area"	**T. Vrijenhoek**

10:30 Coffee Break

11:00 - 13:00

"Analysis pipeline, variant database and lims for exome sequencing data"	**T.M Strom**
Data exchange/access for large scale projects	**T. Meitinger**
Submitting medical sequencing data to an EBI database	**N. Kurbatova**

13:00 Lunch

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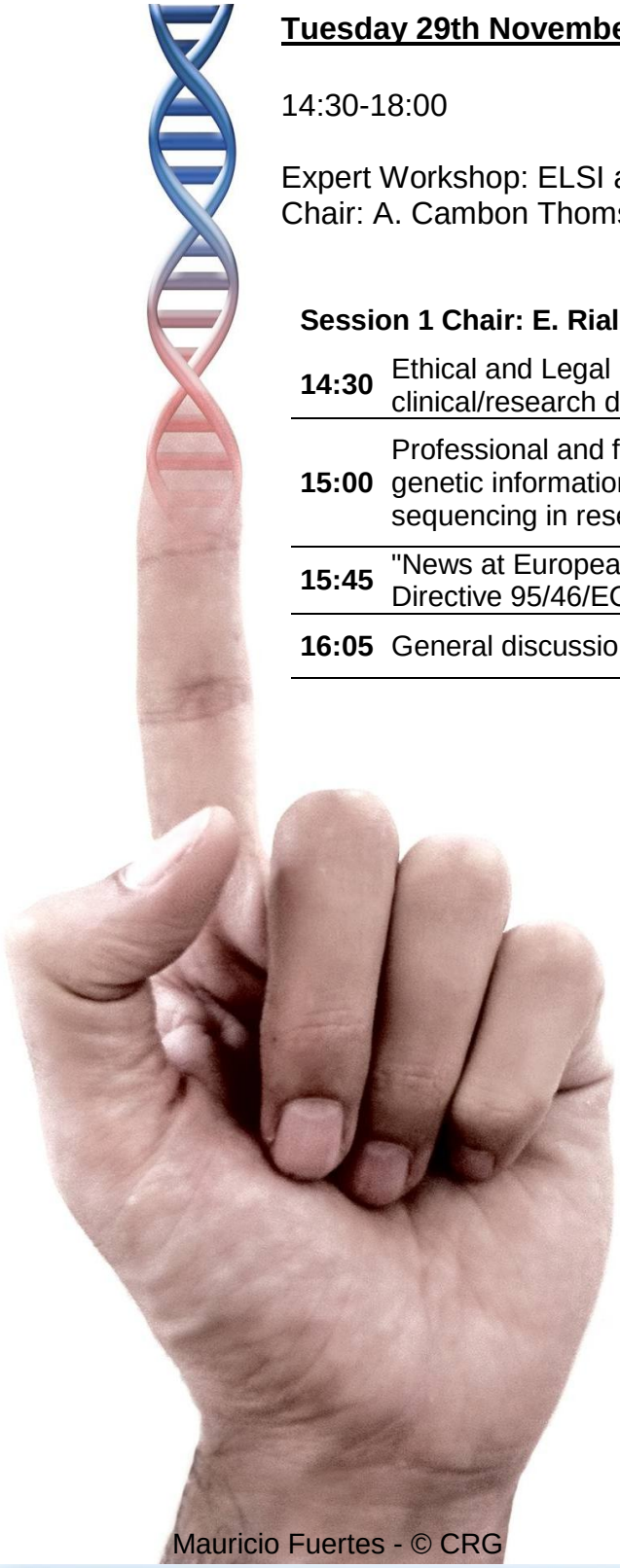

Mauricio Fuertes - © CRG

Tuesday 29th November 2011

14:30-18:00

Expert Workshop: ELSI and NGS
Chair: A. Cambon Thomsen

Session 1 Chair: E. Rial Sebbag

14:30	Ethical and Legal background on sequencing in various clinical/research domains	**H. Howard**
15:00	Professional and family attitudes rearding large scale genetic information generated through next generation sequencing in research	**J. Miller**
15:45	"News at European regulation level: Update on the Directive 95/46/EC, Data Protection Directive"	**G. Chassang**
16:05	General discussion	

16:15 Coffee break

Session 2 Chair: A. Blasimme

16:40	No man is an island. Whole genome sequencing in Direct to consumer	**A. Soulier**
17:10	Discussion: policy guidelines regarding sequences proposed directly to consumer	
17:45	Concluding remarks	**A. Cambon Thomsen**

20:30 Dinner at Brasserie Flo les Beaux Art
1 Quai de la Daurade
Tel: +33 5 61 21 12 12
Access details p.14

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List of chairpeople and speakers:

Emmanuelle Rial-Sebbag - Lawyer-, Graduate in health law (Faculty Bordeaux), Ph.D in Health Law (mention very honourable, University Paul Sabatier Toulouse). She is working since 2000 at the INSERM Unit 1027 in Toulouse (Epidemiology and public health analysis: risks, chronic diseases and handicaps) in the team Genomics, biotherapy and public health: interdisciplinary approach (Dir: Anne Cambon-Thomsen) as a permanent researcher in health law and bioethics. She is an Associate lecturer in bio-law and bioethics at the University of Medicine in Toulouse (Purpan). She is involved in several research projects at National, European and International level, on the topics of biobanking, innovative therapies and biomedical research involving human beings and direct-to-consumer genetic tests. She is responsible for several teaching and educational sessions especially on the ethical and legal aspects of biomedical research involving human, patients? rights regarding biobanking. She is actually developing a research on the Governance of Research in biotechnology and the role played by regulations at national and European level.

Alessandro Blasimme studied political philosophy as an undergraduate and bioethics as a graduate student at "La Sapienza" University of Rome, Italy. He is a PhD candidate at the European School of Molecular Medicine (SEMM - IEO European Institute of Oncology - University of Milan, Itlay) where he is completing a dissertation on the governance of stem cell clinical research. His interests focus on the ethical and political dimension of the life sciences and biomedical innovation, as well as on the epistemological foundations of molecular biology.

Dr. Heidi Carmen Howard is a postdoctoral researcher at the Centre for Biomedical Ethics and Law at the Katholieke Universiteit Leuven (Leuven, Belgium). She is also an invited researcher at the Centre of Genomics and Policy at McGill University (Montreal, Canada). Dr. Howard received her undergraduate and doctoral degrees in Biology from McGill University (Montreal, Canada) where she trained with Dr. Kurt Sittmann and Dr. Guy Rouleau respectively. The focus of her Ph.D. was neurogenetics and it culminated in the cloning of the gene for ACCPN. As a CIHR and Juan de la Cierva postdoctoral fellow she then continued her training in psychiatric genetics at the Centre for Genomic Regulation with Dr. Xavier Estivill (Barcelona, Spain) and at the Douglas Hospital with Drs. Ridha Joober and Howard Steiger (Montreal, Canada). With an Erasmus Mundus fellowship, she completed the Erasmus Mundus Master of Bioethics programme in 2008 and has since been working on the ethical, legal and social issues (ELSI) related to genetics and genomics. As the recipient of a Marie Curie research fellowship (2009-2011), Dr. Howard is presently training in bioethics with Drs. Herman Nys and Pascal Borry at the K.U.Leuven. Much of her work in bioethics, to date, has focused on the ELSI of direct-to-consumer (DTC) genetic testing but she is also studying ethical aspects pertaining to public health genomics, biobanking, pharmacogenomics and genetics education

Jane Miller: After obtaining a BSc in Anatomy & Pathology, she worked as a clinical embryologist for 7 years. She then moved into research, mainly producing transgenic mice through microinjection, and also embryonic stem cell culture for blastocyst injection. She then returned to university for an MPhil in philosophy and ethics and a PhD thesis entitled 'Health and the Human Genome Project'. This lead to qualitative research in Wales on the introduction of genetic testing for Familial Hypercholesterolaemia, where she interviewed participating individuals. Her current position is with Inserm in Toulouse where she is working on the ELSI part of GEUVADIS.

Gauthier Chassang studied in the University of Law of Toulouse Capitole (UT1) and since 2009, he holds a Master degree in European, International and Compared Law. Working in the field of health law and ethics of genetics and new technologies at the French National Institute of Health and Medical Research, Institut National de la Santé et de la Recherche Medicale (Inserm) he participated / is participating to several European projects (Ga²len; BBMRI; PHGEN II).

Alexandra Soulier holds master degrees in philosophy, political science and economy. She has been working in the French National Institute of Health and Medical Research since 2009 where she has participated to the ethical management of European projects involving biotechnologies. She is completing a philosophical dissertation about "biological citizenship" as promoted in biobanking.

ABSTRACT BOOK
Oral presentations

Natalja Kurbatova
Monday 28th November 17:00

"Ontology development for annotating high throughput sequencing experiments

The Experimental Factor Ontology (EFO) was initially developed in order to annotate experimental data in ArrayExpress and in other databases at EBI. The methodology used when developing the EFO involves transforming terms in an experimental variable vocabulary into an ontological representation, and forming classes and relationships between those classes. In close collaboration with EBI European Nucleotide Archive (ENA) a number of terms specific for high-throughput sequencing experiments have been added to EFO. New classes and groupings have been created, and synonyms, external references, axioms and definitions have been added or revised in order to make EFO terms easily accessible

Some examples of terms specific for high throughput sequencing experiments are

“library strategy”, “library source” and “sequencing file type”. EFO is used for data annotation and submission in both the ArrayExpress and ENA databases.

High-throughput sequencing experiments in EBI archives

High-throughput sequencing data is handled at EBI in three major repositories: ArrayExpress, European Nucleotide Archive (ENA) and European Genome-phenome Archive (EGA).

The ENA accepts data generated by high-throughput sequencing platforms such as Roche 454, Illumina Genome Analyzer and ABI SOLiD. ENA works in close collaboration with the NCBI and DDBJ as part of the International Nucleotide Sequence Database Collaboration (INSDC). All submitted public data is exchanged between the partners on a daily basis. ENA stores sequence read data and technical meta-data describing how a sequencing experiment has been processed, for example base coordinates, library layout (single or paired) and spot length. Raw data can be submitted in different formats, but all data are converted into fastq files in the archive. As a result, users can access both originally submitted raw data and processed raw data in fastq format.

ArrayExpress, on the other hand, is a database of functional genomics experiments including those based on high-throughput sequencing technology. It stores experiment meta-data including description of samples, variable factors, and experimental design, as well as processed data such as mapping files in bam format, and references to the raw data stored in the ENA.

If a submission contains human identifiable data, it will go through the European Genome-phenome archive which provides secure storage and works with data access committees for controlled access when that is required.

At the moment we provide two possibilities for high-throughput sequencing data submitters:

1) Submit data into ArrayExpress in MAGE-TAB format. In such a case the raw data will be passed on to ENA/EGA, while metad-data and processed data will be stored in ArrayExpress.

2) Submit data into ENA, get accession numbers for study and submit meta-data together with processed

data into ArrayExpress including ENA accessions into MAGE-TAB file.

Two examples of high throughput sequencing experiments submitted to ArrayExpress archive can be accessed at:

http://www.ebi.ac.uk/arrayexpress/browse.html?keywords=E-MTAB-730&expandefo=on

http://www.ebi.ac.uk/arrayexpress/browse.html?keywords=E-MTAB-440&expandefo=on

The described data flow where ArrayExpress stores meta-data and processed data and ENA stores the raw sequence read data fully supports the MINSEQE (Minimum information about a high-throughput SeQuencing Experiment) standard. MINSEQE describes guidelines for the minimum information that should be reported about high-throughput sequencing experiments to enable unambiguous interpretation (see http://www.mged.org/minseqe/).

Data generated in Geuvadis project such as protocols comparison in different labs can be easily submitted into EBI archives.

Gene Expression Atlas and high-throughput sequencing data pipelines.

In order to be able process high-throughput sequencing data automatically and obtain mRNA/microRNA expressions, two pipelines have been developed and are currently being tested and evaluated:

KRAKEN for microRNA high-throughput sequencing experiments;

ArrayExpressHTS for RNA-seq experiments designed for expression profiling of known transcripts.

The methodology and standards developed and evaluated by Geuvadis will be compared with results from our present pipelines, and we aim at implementing the findings of Geuvadis as individual pipelines as well.

Tuuli Lappalainen
Monday 28th November 17:20

"Epistatic selection between coding and regulatory variation in human evolution and disease" Tuuli Lappalainen, Stephen B Montgomery, Alexandra C Nica, The MuTHER Consortium, Emmanouil T Dermitzakis

Interaction between genetic variants has been highlighted as an important mechanism underlying phenotypic variation. In this study, we show that the variation in the human genome has been shaped by local epistasis, with cis regulatory variation modifying the penetrance of putatively deleterious protein-coding variants. We first analyzed 1000 Genomes pilot populations with gene expression data from arrays and RNA-sequencing. We observed an underrepresentation of functional coding variation on the higher expressed regulatory haplotype, which suggests stronger purifying selection against deleterious coding variants with increased penetrance. Furthermore, the frequency spectrum and impact size distribution of common regulatory variants (eQTLs) is shaped in order to minimize the accumulation of deleterious coding mutations on the higher expressed haplotype. Thus, the overall spectrum of functional variation appears to be optimized by evolution to maximize fitness by avoidance of deleterious local epistasis. Interestingly, 358 common disease associated eQTLs from the MuTHER dataset show a pattern exactly opposite to the general pattern. These genes carried signals of increased penetrance of rare coding variants, with excess of variation in the higher expressed haplotype and a frequency distribution predisposing to deleterious epistasis. Altogether, our results indicate that regulatory variation not only

changes gene expression levels but also affects the penetrance of coding variants, which also implies that knowing the regulatory context is important for predicting the functional impact of coding variants. Characterizing these joint effects may help to understand functional mechanisms behind genetic associations to human phenotypes – including both Mendelian and common disease.

Terry Vrijenhoek
Tuesday 29th November 10:00

"Next-generation sequencing into the diagnostic area"

Many academic hospitals in the Netherlands have made initial preparations for application of next-generation sequencing (NGS) in routine diagnostics. While NGS has thus far been used as a research tool, patients in the Netherlands are among the first to receive a diagnosis based on data from exome or large gene-set sequencing.

In order to maintain a high-quality level of care, the Centre for Genome Diagnostics develops guidelines and standards of care for NGS-based diagnostics in the Netherlands. It has started a pilot study – project CARDIO – comprising ten patients with cardiovascular disease. All patients have already been included into a routine diagnostic and counseling trajectory, and are thus not dependent on the results from the pilot study for their diagnosis. DNA samples and medical records of all patients are being collected, and subsequently shared with all clinical centres for clinical assessment, NGS-based testing, diagnosis and counseling. Each partner is free in choosing their preferred approach in any of these steps, provided that they carefully describe and share the full diagnostic process and the outcomes of each step in that process. The centres work together and share results in a Virtual Diagnostic Department, which should develop into a permanent test environment for novel diagnostic applications and serve as an education tool for medical students.

Based on the results of project CARDIO guidelines and standards can be developed on the major challenges in medical sequencing, such as required infrastructure, data analysis and interpretation, and information to patients. With the collective efforts from researchers, clinicians, ethicists and legal advisors, as well as representatives from platform and service providers, the Centre for Genome Diagnostics will develop a sustainable framework for next-generation diagnostics.

Tim M Strom
Tuesday 29th November 11:00

"Analysis pipeline, variant database and lims for exome sequencing data"

Thomas Wieland, Sebastian H. Eck, Elisabeth Graf, Anna Benet-Pagès, Thomas Meitinge1, Tim M. Strom

Enrichment techniques for targeted sequencing of coding regions are currently applied to identify rare variants. We developed a pipeline to analyze exome sequencing data. The pipline is integrated in a LIMS systems and a database to store all variant data. The pipeline is a combination of Perl scripts and public available software packages (BWA, SAMtools). Annotation includes type of mutation, presence in dbSNP, HGMD and the 1000 Genomes data as well as functional impact on the corresponding protein.

The database can be queried through a web interface using standard queries. Exomes of individuals with other diseases are used as controls. The LIMS is used to organize the workflow from library preparation to completed analysis. Barcoded tubes are used for sample tracking.

We applied the analysis pipeline to ~600 exomes. Sequencing of 10-11 GB results in a read depth of at least 20 for ~90% of the sequences. The pipeline calls ~21,000 coding variants. Approximately 10,000 of these are non-synonymous variants, ~150 variants affect canonical splice sites and ~400 are coding indels. We quantified the amount of new, putatively deleterious variants listed in the Human Gene Mutation Database (HGMD), and assessed the frequency of literature-annotated disease mutations. We identified 46 new heterozygous nonsense variants. 204 of HGMD annotated mutations (~0.75%) had a frequency of >2% in our samples. After subtracting these mutations with an unlikely high frequency, the carrier burden of recessive mutations from HGMD in these genes is between 0 and 12 per individual (average 3.6).

Additionally, we determined the distribution of non-synonymous variants per gene for OMIM and non-OMIM genes. The number of variants are normalized to 1000 aa. The normalized values do not differ between OMIM (15) and non-OMIM (16) genes. Genes with an excess of variants are identified and can be excluded from the analysis of rare variants.

Gauthier Chassang
Tuesday 29th November 15:45

"News at European regulation level: Update on the Directive 95/46/EC , Data Protection Directive"

Jane Miller, Emmanuelle Rial-Sebbag, Gauthier Chassang, Anna Pigeon, Velizara Anastasova, Anne Cambon-Thomsen

This Directive, implemented in 1995, is currently undergoing review by the Commission. The amendments to the Directive are expected to be published in 2012 A public consultation was launched in July 2009 via the EU website 'Your Voice in Europe' and closed in December 2009. It received 168 responses from individuals, business organisations & associations, and public authorities. A 'Summary of replies to the Public Consultation about the future legal framework for protecting personal data' was published in November 2010 and reviews were also undertaken by the Commission's relevant working parties and committees. The main axes of the revision concern *The right to be forgotten, Transparency, "Privacy by default", Protection regardless of data location.* A report from the European Parliament that supported the reforms of the Directive was received in July 2011 and the Commission is utilising all these inputs to redraft the legislation. Especially a working group known as "*Art.29* Data Protection Working *Party"* has made several proposals, such as in July 2011 the Opinion 15/2011 on the definition of consent. A communication issued in July 2011 stated that their aim was to 'reduce the current fragmentation of the EU legal framework, reinforce the internal market dimension of data protection and strengthen the rights of individuals.' It also outlined that an 'important part of the reform will be improving data security.' The sections of the Directive relevant to biomedical research will be outlined, specifically with regard to genetic and sequencing data, and the possible impact that the proposed changes may have on these issues.

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Poster abstract:

"Professional and family attitudes regarding large scale genetic information generated through next generation sequencing in research"

Cambon-Thomsen, A ; Miller, J ; Soulier, A ; Bertier, G ; GEUVADIS consortium

The use of high throughput techniques for genomic sequencing creates large amounts of genetic data that can give rise to new medical, ethical, legal and social questions, especially with regard to privacy and confidentiality and the creation of health related information which may, or may not be, clinically useful, with a blurring of the boundary between a clinical and research context. Although most of the traditional ethical/ legal frameworks that have been developed in relation to genetic research continue to be applicable, some new aspects arise with sequencing technology e.g. sources of samples; type of consent; the scope and duration of studies; a right to withdraw; whether it is a family decision; sensitivity of, and access to, the data; return of results; and issues relating to direct to consumer tests.

In order to gain insights into such issues, group discussions and questionnaires were conducted with researchers and non-researchers in 5 countries in the context of 3 EU funded projects (GEUVADIS, CAGEKID, ESGI).

The results of the questionnaires and discussion groups generally underlined the importance of clear and transparent information and an understanding of the aims of the study. It is also clear from the results that these issues should be dealt with at an early stage of the research process by all interested parties.

This poster will outline the findings from the questionnaires.

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Sunday 27th November 2011

20:30 Welcome Dinner at the Hotel Restaurant: La Ripaille

Menu:

Season's raw vegetables plate

***

Pork "Paupillette" with prunes

***

Chocolate Mousse

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Monday 28th November 2011

20:30 Dinner at Restaurant **La Bohème.***
3 rue Lafayette
10 mins walk from the Hotel
Metro: Capitole
+33 5 61 23 24 18

Menu:

Confit gizzard and smoked magret salad
***
Home-made Cassoulet
or
The chef's fish choice
***
Chocolate cake and custard
or
'île flottante'
or
'crème brûlée'
***
AOC wine
Coffee

Access Details:

To walk from the hotel (10 mins):

1. Head **southwest** on **Rue Bayard** toward **Rue de Belfort** 350 m

2. Turn left onto **Rue d'Alsace Lorraine** 300 m

3. Turn right onto **Rue Lafayette.** Destination will be on the right 130 m

See map on the next page

Grand Hôtel d'Orléans - **Salle Riquet**
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Monday 28th November 2011

20:30 Dinner at **Restaurant La Bohème.***
3 rue Lafayette
10 mins walk from the Hotel
Metro: Capitole
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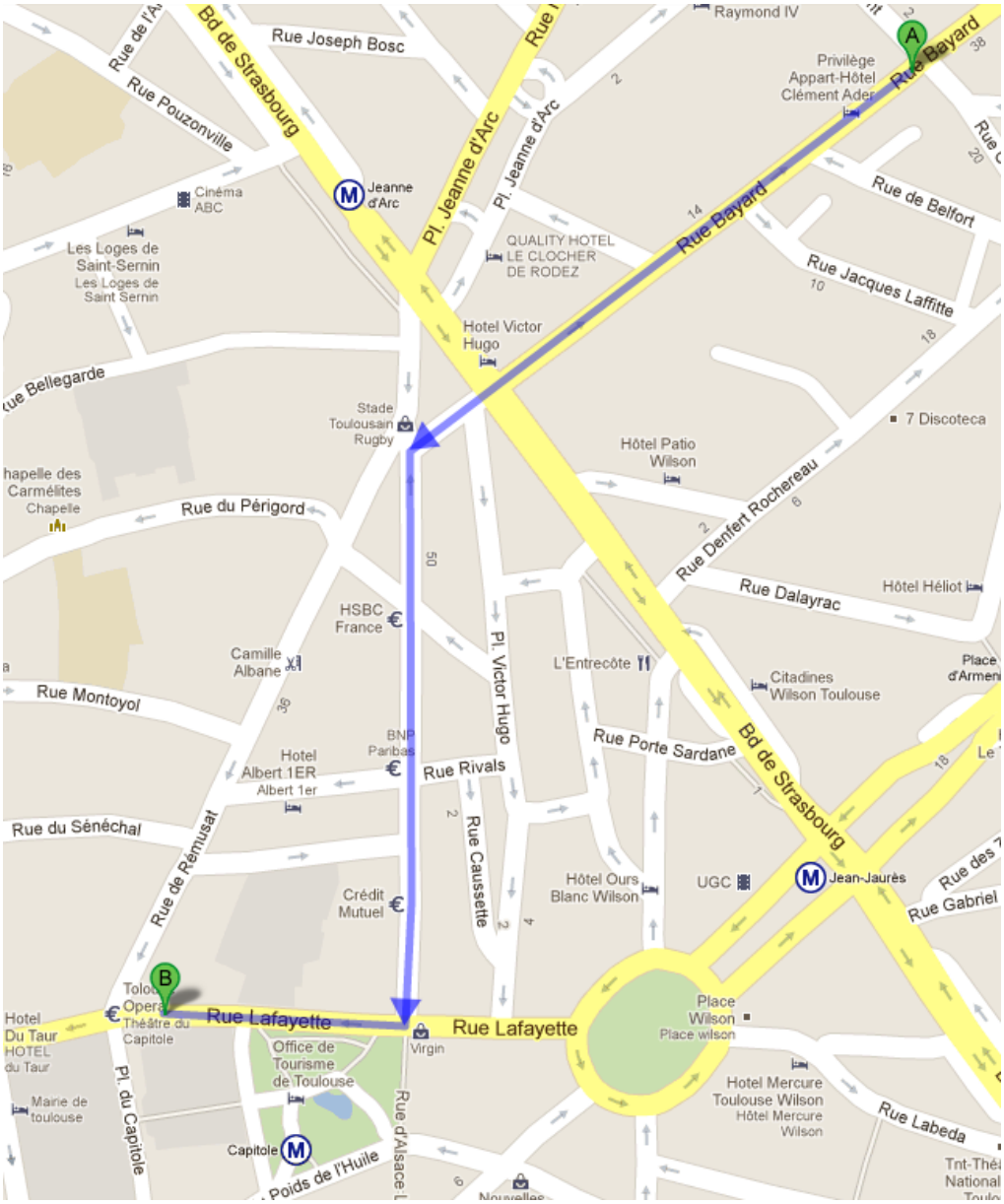

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Tuesday 29th November 2011

*20:30 Dinner at **Brasserie Flo les Beaux Art.***
1 Quai de la Daurade
20 mins walk (or 3 Metro stops) from the hotel
Metro: Esquirol
Tel: +33 5 61 21 12 12

Menu:

White wine kir
***
Duck foie gras, fruit chutney
or
Crispy raw milk Cantal cheese, mixed salad
***
Norvegian salmon plancha, mixed vegatable and "Nantais" butter
or
Rumsteck, pepper sauce, gratin dauphinois
***
Crispy and smooth choclate cake
or
burnt vanilla cream
***
1/4 Côtes de Gascogne red or white wine
1/2 mineral water
coffee

See map on the next page

Access Details:

To walk from the hotel (20mins):

1. Head **southwest** on **Rue Bayard** toward **Rue de Belfort** 350 m
2. Turn left onto **Rue d'Alsace Lorraine** 800 m
3. Turn right onto **Pl. Esquirol**160 m
4. Continue onto **Rue de Metz**. 210 m
5. Continue onto **Pl. du Pont Neuf.** 57 m
6. Turn right onto **Quai de la Daurade** Destination will be on the right 10m

Or take the metro at **Marengo sncf** (train station).

Take lign A direction Basso Combo.

Stop at metro Esquirol

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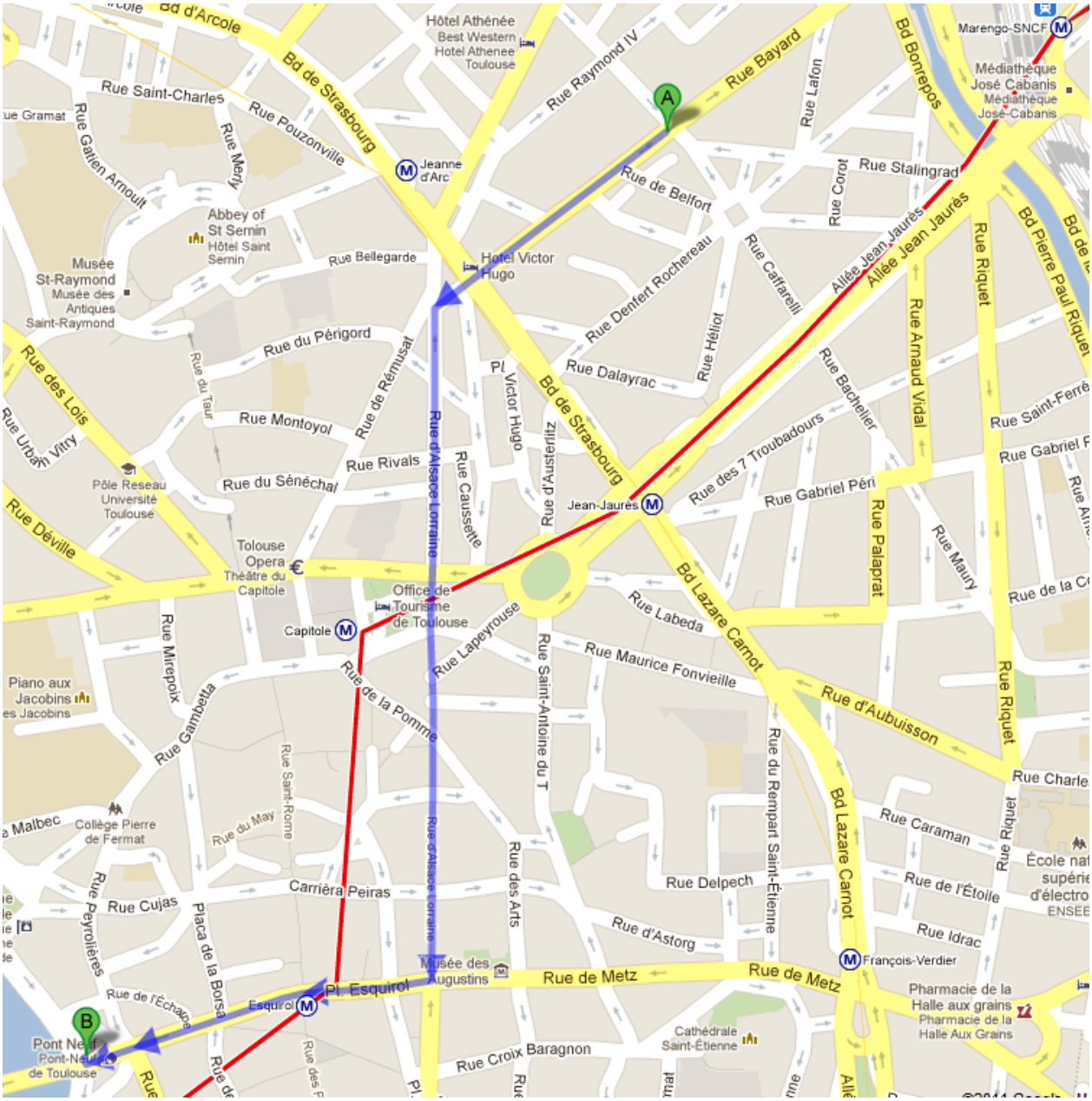

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List of participants

1	Gabrielle Bertier	CRG
2	Estivill	CRG
3	Tuuli Lappalainen	UNIGE
4	Thomas Meitinger	HMGU
5	Tim M Strom	HMGU
6	Jörg Hager	CEA-CNG
7	Ralf Sudbrak	MPIMG
8	Marc Sultan	MPIMG
9	Michael, Sammeth	CNAG
10	Ivo Gut	CNAG
11	Ann-Christine Syvänen	UU
12	Mathias Brännvall	UU
13	Philip Rosenstiel	CAU
14	Joris Veltman	RUNMC
15	Terry Vrijenhoek	RUNMC
16	Henk, Buermans	LUMC
17	GertJan van Ommen	LUMC
18	Beatriz, Sobrino	USC
19	Natalja Kurbatova	EBI
20	Jane Miller	Inserm
21	Anne, Cambon-Thomsen	Inserm
22	Rial-Sebbag Emmanuelle	Inserm
23	Chassang Gauthier	Inserm
24	Alexandra Soulier	Inserm
25	Alessandro Blasimme	Invited speaker
26	Heidi Howard	Invited speaker
27	Jonathan Mangion	Life Technologies
28	Ada Hamosh	JHU
29	Pui-Yan Kwok	SAB